The results suggest that aromatic residues do not increase the binding affinity of bupivacaine to Kv channels. Rather, the affinity decreases. The Kd value for the wildtype channel is 300 μM, for the V473F channel 550 μM and for the P474F channel 740 μM. Thus, aromatic residues seem not to be necessary for high-affinity local anaesthetic binding to voltage-gated ion channels. The specific role of aromatic residues in Nav and hERG channels seems thus related to specific structural constraints in these channels.

2890-Plat
A Regulator for Eag Family Channels
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Ether-a-go-go (Eag) family channels, which include hErg1, are voltage-gated K^+ channels that are important in cardiac and neural function. From the genetic sequence of this family of channels, we identify two probable ligand-binding sites based on their similarities with well-characterized ligand binding domains. The first putative ligand-binding domain is in the carboxy-terminal region and shares sequence similarity with the cyclic nucleotide-binding domain of cyclic nucleotide-gated (CNG) channels. Yet, this binding domain of Eag family channels lack a critical arginine required for cyclic nucleotide binding, and channel gating was not altered by cAMP or cGMP. The second potential ligand-binding site is a Per-Arnt-Sim (PAS) domain in the amino-terminal region. High conservation of these putative binding domains amongst all Eag family channels indicates their functional importance. We therefore categorize these channels as orphan receptors. We reasoned that a chemical screen of cellular metabolites will lead us to physiologically relevant channel-regulators. Using a novel, high through-put screen of the ''Fragments of Life'' chemical library of metabolites and metabolite-like compounds (deCODE Biostructures) and inside-out patch-clamp recording, we have identified regulators of Eag family channels. We identified six regulators that cluster into four chemical families. One indole, and one indole-like compound were increased Eag channel opening at hyperpolarizing potentials. Indoles are particularly interesting because of their structural similarity to purines, the core of the cyclic nucleotides that bind to and regulate CNG channels. In contrast to indoles, compounds from the flavonoid family strongly inhibited Eag current. These results indicate that metabolites regulate Eag family channels and may lead us to physiologic channel regulators.

Platform BC: Cardiac Electrophysiology

2891-Plat
The Vena Cava Is Pacing The Embryonic Heart
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The mechanism and location of the pacemaker in the embryonic heart is highly controversial due to the lack of physiological *in vivo* recordings. Here we report fluorescent macroscopic *in vivo* recordings of embryonic hearts (E12.5-E14.5) from mice with cardiac expression (α-MHC or Cx40 promotor) of the Ca^{2+} sensor GCaMP2.
Initial observations from the ventral surface showed regular uniform Ca^{2+} transients in the atrium, ventricle and a structure below/behind the right atrium that preceded every atrial activation. To better understand the origin of these Ca^{2+} transients we established a dorsal preparation leaving the heart and veins intact. Ca^{2+} transients activated in the region of the putative sinus node, propagated bidirectionally along the superior right and in a u-shaped pattern into the coronary sinus and left superior vena cava, and conducted faster (24 ± 4 mm/sec) than the atria (15 ± 2 mm/sec; n=5; $p<0.02$); we have termed this the ''streak''. In most hearts the streak fired before every atrial activation with a delay of 79 ± 10 ms (n=12); variations in this delay was not dependent on the heart rate (104 ± 15 bpm, n=12). Some hearts showed 2:1 coupling and in others only the streak fired at 178 ± 39 bpm (n=5). The streak contracted, could be electrically paced and spontaneous local field potentials were recorded as sharp spikes at the onset of Ca^{2+} transients. In some experiments we observed a diastolic Ca^{2+} increment just prior the field potential, in line with the proposed role of Ca^{2+} oscillations for the initiation of pacemaking in embryonic heart cells. The spontaneous activity of the streak prior to atrial activation, higher intrinsic frequency of this region, and diastolic Ca^{2+} release establish the pacemaker function of the streak. Thus, cardiomyocytes within/on the vena cava walls are involved in murine embryonic heart pacemaker activity. Values: mean ± SEM.

2892-Plat
Increased Vulnerability To Atrial Fibrillation Under Vagal Hyperinnervation Associated With Vasoactive Intestinal Polypeptide'S Release In Dog'S Atrium
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Increased vagal tone promotes atrial fibrillation (AF). Vasoactive intestine polypeptide (VIP, a neural transmitter co-released with acetylcholine) was shown to shorten atrial refractory periods. AF was reportedly associated with VIPoma, a rare tumor secreting excessive VIP. Yet, the effects of VIP on atrial electrophysiology remain unclear. **Methods:** Canine left atria were isolated with intact coronary perfusion. Programmed stimulation (300 ms drive cycle length followed by up to 4 extrastimuli) was used to assess AF induction. Action potential duration (APD) at 500 ms pacing was recorded with optical mapping system. Potassium currents (I_{Ks}) were recorded with patch-clamp techniques. Immunohistochemical staining for VIP receptors was performed on atrial tissue/myocytes. **Results:** AF was induced in 1 of 6 atria at baseline but 5 of 6 during 1 μM VIP perfusion ($p=0.021$) and 2 of 6 after 15 min washout. VIP shortened APD and increased inhomogeneity in a dose-dependent manner at 0, 0.1, 1.0, 10 μM and washout (n=13): APD_{75} was 134.61 ± 5.36, 116.31 ± 6.80, 117.50 ± 7.86, 100.71 ± 8.73 and 124.50 ± 4.84 ms; standard deviation of APD_{75} was 15.83 ± 1.55, 15.59 ± 2.33, 21.58 ± 3.57, 25.76 ± 1.16 and 18.67 ± 1.62 ms, respectively ($p<0.05$). VIP (1 μM) increased I_{Ks} current density (14.5 ± 9.5%, n = 9, $p<0.01$). Staining on isolated atrial myocytes revealed the expression of VIP receptor 1 and 2 was highly variable among cells and tissue staining showed spatial heterogeneity. **Conclusions:** VIP shortens APD and increases APD spatial inhomogeneity that could lead to increased AF vulnerability. Enhanced I_{Ks} and heterogeneity of receptor expression may contribute to these effects.

2893-Plat
Ablation of Protein Kinase A or Calmodulin Kinase II Phosphorylation Sites on Phospholamban Confer Arrhythmia Resistance in Sinoatrial Nodal Pacemaker Cells
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Phospholamban (PLN) is a negative regulator of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCa). PLN is phosphorylated by Protein Kinase A (Ser16) or Calmodulin Kinase II (Thr17). These phosphorylations reduce the inhibitory effects of PLN on SERCa. Phosphorylation at PLN Ser16 and Thr17 are important for catecholamine effects on excitation-contraction coupling in ventricular myocytes, but their role in sinoatrial nodal (SAN) cells is unknown. We isolated SAN cells from wild type (WT) controls and from transgenic mice where Ser16Ala or Thr17Ala PLN mutants are expressed in lieu of WT PLN. We recorded spontaneous action potentials by perforated patch clamp at 35 ± 1°C. SAN cell automaticity rates ('beats'/min) were not significantly different between Ser16Ala, Thr17Ala or WT at baseline: WT (n=18) 269 ± 12; T17A (n=9) 264 ± 23; S16A (n=16) 256 ± 17. No early (EAD) or delayed (DAD) afterdepolarizations were observed at baseline. EADs and DADs were induced by isoproterenol (1-5 μM) in WT SAN cells (cells with afterdepolarizations/total cells tested: 10/13) but not in Ser16Ala (0/6) and Thr17Ala (0/5) SAN cells ($p<0.01$ in both cases compared to WT). These results suggest that catecholamine induced EADs and DADs require phosphorylation of PLN Ser16 or Thr17 and highlight the importance of the intracellular Ca^{2+} cycling machinery for determining SAN cell vulnerability to adverse effects of catecholamine stimulation.

2894-Plat
Arrhythmogenic potential of activated fibroblasts
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Many cardiovascular disorders including ischemic heart disease and heart failure are associated with extensive fibrosis. A critical event in the development of cardiac fibrosis is the transformation of fibroblasts into an active fibroblast phenotype or myofibroblast. Fibroblasts isolated from healthy hearts and grown under standard tissue culture conditions start expressing the myofibroblast marker α-SMA 24-48 hours after isolation. These cells have been referred to as myofibroblasts. However, there is evidence indicating *in vitro* phenotypic changes due to culture conditions do not fully replicate the *in vivo* activation process.